

# UNCLASSIFIED

AD NUMBER
ADB266333
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies only; Proprietary Information; Aug 2000. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, MD 21702-5012
AUTHORITY
USAMRMC ltr, 8 Jan 2003

THIS PAGE IS UNCLASSIFIED

AD\_\_\_\_\_

Award Number: DAMD17-98-1-8183

TITLE: Tagging of Breast Tumors for Excision and Specimen  
Radiography and of Sentinel Nodes for Ultrasound-Guided  
Localization Using Novel Particulate Agents

PRINCIPAL INVESTIGATOR: Robert F. Mattrey, M.D.

CONTRACTING ORGANIZATION: University of California, San Diego  
La Jolla, California 92093-0934

REPORT DATE: August 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government  
agencies only (proprietary information, Aug 00). Other requests  
for this document shall be referred to U.S. Army Medical Research  
and Materiel Command, 504 Scott Street, Fort Detrick, Maryland  
21702-5012.

The views, opinions and/or findings contained in this report are  
those of the author(s) and should not be construed as an official  
Department of the Army position, policy or decision unless so  
designated by other documentation.

20010521 089

## NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

### LIMITED RIGHTS LEGEND

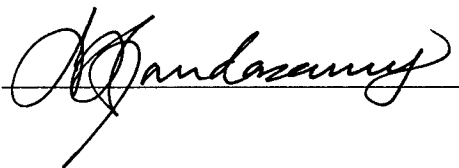
Award Number: DAMD17-98-1-8183

Organization: University of California, San Diego

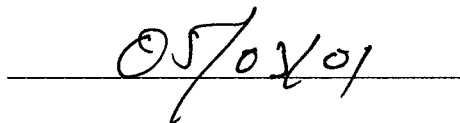
Location of Limited Rights Data (Pages):

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.



---



---

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY (Leave blank)</b>		<b>2. REPORT DATE</b> August 2000	<b>3. REPORT TYPE AND DATES COVERED</b> Annual (1 Aug 99 - 31 Jul 00)	
<b>4. TITLE AND SUBTITLE</b> Tagging of Breast Tumors for Excision and Specimen Radiography and of Sentinel Nodes for Ultrasound-Guided Localization Using Novel Particulate Agents			<b>5. FUNDING NUMBERS</b> DAMD17-98-1-8183	
<b>6. AUTHOR(S)</b> Robert F. Mattrey, M.D.				
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> University of California, San Diego La Jolla, California 92093-0934  <b>E-MAIL:</b> rmattrey@ucsd.edu			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b> This report contains colored photos				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Aug 00). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.				<b>12b. DISTRIBUTION CODE</b>
<b>13. ABSTRACT (Maximum 200 Words)</b>  <b>Purpose:</b> Year 1, we identified a radiopaque tumor-marking agent and optimized the dose. This year we worked on coloring that agent and sentinel node detection. <b>Scope:</b> We could not color the radiopaque perfluorocarbon (PFC) emulsion, but successfully suspended India Ink within it. The optimum dose was then injected in tumors. We followed the black tract to locate/remove the tumor at 72-hours. The resected specimen was then radiographed. We worked on sentinel node detection with another agent. <b>Results:</b> The black color identified the tumor in all rabbits. The radiopaque agent was visible within the tumor at 72-hours. Since it is difficult to color PFC emulsions, we abandoned the non-radiopaque PFC emulsion approach to locate the sentinel node. We identified a lipid emulsion where Sudan Black is dissolved within the vesicles. <b>Significance:</b> We have marked both the tumor with a radiopaque agent for specimen radiography and the tract to guide the surgeon to the tumor. We have identified a blue lipid emulsion that should have a longer dwell time in the lymphatics and a greater ability to localize in the sentinel node. The final year we will test the blue emulsion and compare its pharmacokinetics and sentinel node enhancement ability relative to the water-soluble blue dye.				
<b>14. SUBJECT TERMS</b> Breast Cancer			<b>15. NUMBER OF PAGES</b> 11	
			<b>16. PRICE CODE</b>	
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

## Table of Contents

Cover.....	
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	5
Appendices.....	6

## Introduction

Our grant seeks solutions to two related problems in breast tumor surgery and imaging: (1) provide the ability to mark nonpalpable and non-radiopaque lesions to guide surgery and prove their resection on specimen radiography. (2) Mark and localize the sentinel node. We aimed to accomplish these two goals using perfluorocarbon (PFC) emulsions.

Because it was known that PFC emulsions, that can be radiopaque, remain at the injection site for several days and because it was also known that these PFC emulsions are removed by the local lymphatic circulation and accumulate in macrophages in the draining nodes [1], we aimed to optimize these emulsions and to color them to achieve the 2 goals outline above. In the first year of this award we demonstrated the feasibility of marking tumors with the radiopaque PFC emulsion in a rabbit model. In the second year we refined our technique of marking tumors by tailoring the injection dose to the size of the tumor and site of injection using ultrasound guidance. This allowed us to limit the amount of contrast extravasation as well as optimize the enhancement of the tumor site. Subsequently, we attempted to color the PFC emulsion and tested whether fluorescent labeling was feasible. Neither approach was optimal. We chose to add India Ink to the emulsion to create a black suspension. The black radiopaque agent was then tested to determine its ability to mark non-radiopaque tumors to guide the dissection to the tumor and to assess that the region marked was actually removed on specimen radiography.

## Body of Progress Report

In our last progress report we discussed our results to hypothesis #1: When a radiopaque perfluorocarbon (PFC) emulsion is injected in the lesion it will mark the lesion for days and will allow the confirmation that the marked lesion is contained in the resected specimen on ex vivo radiography.

We were successful at marking the lesions; however, refinement of our technique to minimize extravasation and to limit the agent to the tumor was needed. Since then, we have reported our progress in both an abstract as well as poster presentation at the Department of Defense: Era of Hope Meeting, June 8-12th, 2000 "Marking Mammographically Invisible Tumors for Surgical Guidance and Proof of Resection by Ex-vivo Radiography" (see Appendix).

Our secondary goal in tumor marking was to color the radiopaque PFC emulsion to provide a tract that the surgeon can follow to the lesion and recognize the lesion when the injection site is reached. We attempted incorporating lipid-based dyes in the PFC, but were unsuccessful as the PFC was not only hydrophobic but also sufficiently lipophobic to accept the dye. Based on calculations of the egg-yolk phospholipid concentration in the emulsion, we concluded that there would be limited fluorescent label that could be incorporated in the emulsifier to allow sufficient signal to be visualized with the naked eye. We elected to follow a different approach. We suspended India Ink in the emulsion at a dose of 0.1ml/ml of emulsion. The experiment was repeated and the results also reported in the poster included in the appendix.

The second major aim of the award is to help localize the sentinel lymph node by improving upon the water-soluble dye. The major weakness of the water-soluble dye is its rapid and unpredictable transit through the lymphatic system following subcutaneous injection. The goal was to produce a visible particulate emulsion. We began experimenting with a non-radiopaque PFC emulsion. Although the emulsion was removed by the lymph vessels, we abandoned this approach following several experiments. The reason for abandoning the PFC

emulsion approach was due to the difficulty we experienced in coloring the radiopaque PFC emulsion. Although we suggested in our proposal that we will use fluorescent labels to provide visual guidance, we sought a solution that would allow direct visualization of the dye under ambient light. Since the goal is to provide visual guidance to the sentinel node, we investigated non-PFC emulsion approaches and have identified an experimental formulation that could serve this function. The agent we identified is a lipid emulsion in which a lipophilic dye has been dissolved. During the final year we should finish comparing its pharmacokinetics to that of the water-soluble dye. Because it is a particulate agent and particulate agents accumulate in macrophages located in lymph nodes, it is possible that this agent will preferentially accumulate in the first draining node, which is the sentinel node of the injection site.

### Key Research Accomplishments

- ⇒ We are able to use ultrasound guidance to inject contrast and mark the implanted tumor and confirm the area of injection with X-ray and CT Scan.
- ⇒ We are able to fill the draining node (sentinel node) with radiopaque contrast and image the node by CT.
- ⇒ We are able to quantify contrast washout from the tumor, degree of leakage, and lymphatic accumulation using region of interest analysis of CT images.
- ⇒ We showed by CT that the radiopaque agent was still visible within the tumor by 72 hours after injection.
- ⇒ We have determined the variables that promoted extravasation and are able to avoid extravasation.
- ⇒ We were successful in suspending India Ink, a black marker, in the radiopaque emulsion and showed that we can mark the lesion for several days in-vivo and upon resection, visualize the dye within the tumor.
- ⇒ We have investigated several approaches for sentinel node visualization. We have identified a likely approach to accomplish this goal. We are investigating this during the last year of the award.

### Reportable Outcomes

A manuscript is being prepared to report the findings described in the poster which is attached to this report as an appendix.

### Conclusions

The results of our project indicate that we are able to mark a tumor under ultrasound guidance with a black dye that is also radiopaque for at least 72 hours. The dye marks the tract to guide tumor resection and marks the tumor for specimen radiography. We have identified a likely agent that can be used in a similar way as the water-soluble dye, but has more favorable pharmacokinetics. We believe that the ultimate goal of our research will eventually yield pre-operative localization and recognition of the sentinel node and allow its resection as an office procedure under local anesthesia.

### References:

1. Wolf GL, Rogowska J, Hanna GK, Halpern EF. Percutaneous CT lymphography with perflubron: imaging efficacy in rabbits and monkeys. *Radiology* 1994;191:501-5

## Appendix

### MARKING MAMMOGRAPHICALLY INVISIBLE TUMORS FOR SURGICAL GUIDANCE AND PROOF OF RESECTION BY EX-VIVO RADIOGRAPHY

S. P. Pinnell, M.D., Y. Kono M.D., L. Diranieh,  
and R. F. Mattrey, M.D.

Department of Radiology  
University of California, San Diego

**Purpose:** The confirmation that lesions seen on mammography have been removed is possible with specimen radiography. This option is not possible when tumors are detected by alternate techniques. We identified a radiopaque perfluorocarbon (PFC) emulsion that was successfully tested in a phase I study for indirect lymphography where it was shown that the agent remained at the injection site for several days [1]. Our primary goal was to determine that this agent when injected in the tumor would remain at the injection site to mark the lesion for specimen radiography. There were 3 secondary aims. The first was to determine if coloring the PFC emulsion would allow surgical guidance to the lesion in order to decrease the inconvenience of needle localization and allow pre-operative localization days before surgery. The second was to determine the maximum volume of the agent that could be injected in the tumor without contaminating the surrounding tissues. The third was to determine if the radiopaque PFC particles would reach the draining lymph nodes to allow the potential of identifying the location of the sentinel node for pre-operative sentinel node localization.

**Methods/Materials:** Vx2 tumor was implanted in one calf of 12 rabbits and both calves in 6 rabbits and allowed to grow for 14 to 22 days. The 12 rabbits with single tumors were used to define the maximum volume that could be injected into the tumor without causing extravasation. To accomplish this, we calculated tumor volume at the time of injection as  $(4/3) \times (\pi) \times (A/2) \times (B/2) \times (C/2)$ ; where A, B, and C are the 3 orthogonal diameters of the tumor as measured by ultrasound. Using ultrasound guidance, a needle was inserted into the tumor center and serial radiographs were obtained following the injection of 0.1ml increments of the PFC emulsion (AF1053, Alliance Pharmaceutical Corp. La Jolla, CA). The volume of the agent at which extravasation occurred was recorded. The linear correlation relating extravasation volume and tumor volume was calculated.

For the remaining six rabbits with 1 tumor in each calf, 0.1 ml of India ink was added to 1 ml of PFC emulsion and 90% of the extravasation volume was injected into one of the two tumors based on the equation defined above. The tumor in the other leg served as control. The tumor, popliteal fossa, and periaortic regions were imaged at 1, 24, 48, and 72 hours with x-ray and ultrasound. At 72 hours, the rabbits were imaged with CT immediately post sacrifice using 3mm slice thickness serially from the tumor to mid retroperitoneum. Immediately following CT, the leg was photographed and the tumor was resected, its size was measured, and the location of the emulsion assessed. The resected leg and the tumor were then radiographed.

**Results:** The radiopaque PFC emulsion was clearly seen on radiographs (Fig. 1). The linear correlation between tumor volume and the volume of PFC that just caused extravasation was  $0.13 \times \text{tumor volume (cm}^3) + 0.49$  ( $r=0.77$ ) (Fig. 2). In 5 of the 6 rabbits injected with the optimal dose, minimal to no extravasation occurred immediately after injection. The single tumor

where extravasation occurred was small in size making needle placement difficult. Although the radiopacity of the tumor diminished slightly it was still visible at 72 hours (Fig. 3). The India ink colored PFC was identified in all lesions at 72 hours (Fig. 4) which aided in tumor localization and excision at necropsy. Specimen radiographs taken following tumor resection showed absence of radiopaque material in the leg and the presence of the radiopaque material in tumor confirming the resection of the marked tumor (Fig 3).

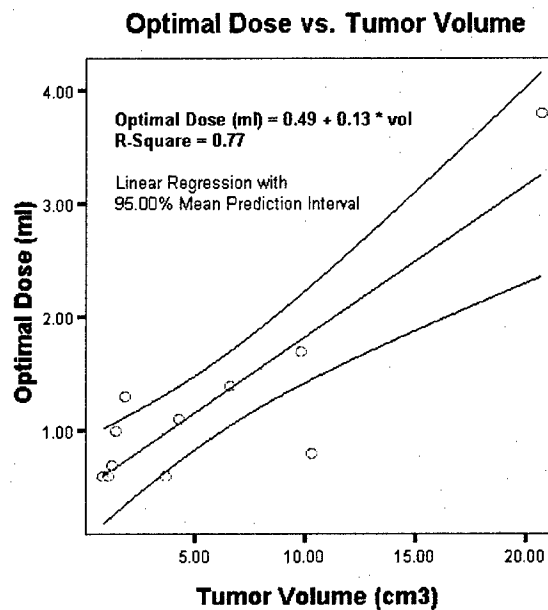
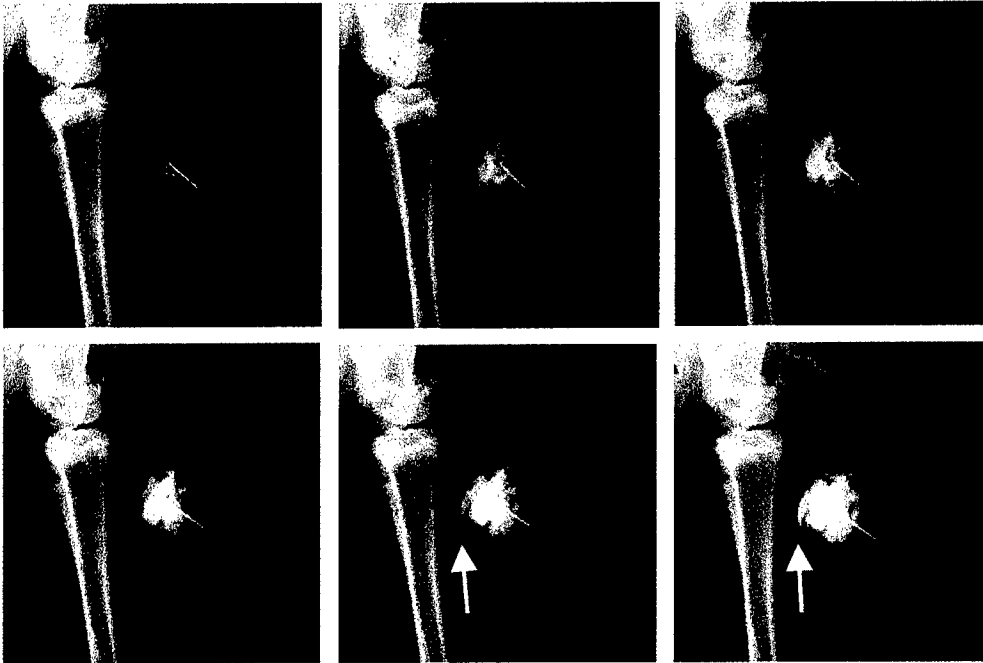
The ipsilateral popliteal node enhanced on CT in 5 of the 6 rabbits by  $37.8 \pm 17.6$  HU relative to the control leg ( $p < 0.05$ ) allowing their easy detection (Fig. 5). This demonstrates that particulates injected in the tumor with minimal or no extravasation can accumulate in the draining nodes. However, in 2 of the 6 rabbits which displayed local popliteal lymph node enhancement, the retroperitoneal lymph nodes enhanced by 96 and 97 HU (Fig. 6) indicating that the agent is not limited to the first draining lymph node. Because PFC emulsions are also visible on ultrasound [2], sonographic enhancement of the popliteal nodes was also observed (Fig. 7).

**Conclusion:** This study showed that the radiopaque PFC emulsion can be colored using India ink. The colored radiopaque emulsion remained at the injection site for at least 72 hours and the addition of India ink aided as a guide to the tumor during resection. It was possible to inject an optimal dose of the agent in this tumor model that did not contaminate the tissues surrounding the tumor. Although the radiopacity in the lesion decreased slightly over 3 days, the agent was still visible at the time of necropsy. Despite a central tumor injection, the particulate contrast agent accumulated in the first node draining the tumor in five of six rabbits and in downstream nodes in 2 of 6 rabbits. Since the agent also has sonographic properties, ultrasound was used to detect local lymph node drainage which may provide pre-operative sentinel lymph node localization in the future.

#### References:

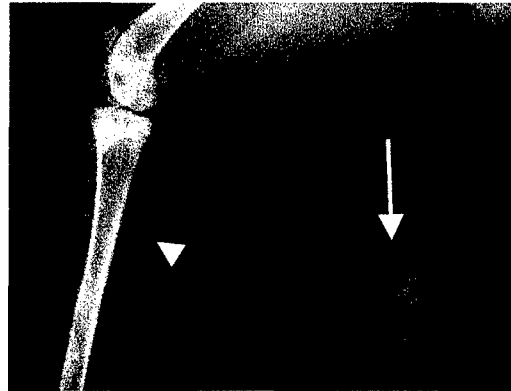
1. Wolf GL, Rogowska J, Hanna GK, Halpern EF. Percutaneous CT lymphography with perflubron: imaging efficacy in rabbits and monkeys. *Radiology* 1994;191:501-5
2. Wrigley RW, Saunders HB, Lim G, Arellano RA, Mattrey RF: Indirect Ultrasonographic Lymphography with Perflubron Emulsion. RSNA Chicago, *Radiology* 1993; 189:285.

**Figure 1:** Serial radiographs of the calf following the injection 0.1, 0.3, 0.5, 0.7, 0.9, and 1.1 ml of AF1053 into the tumor. Note that extravasation (arrow) occurred after 0.9 ml and became more apparent after 1.1 ml.



**Figure 2:** Scatter plot displaying the correlation of emulsion volume that caused extravasation.

**Figure 3:** Specimen radiograph of the leg and tumor (arrow). Note that the tumor is radiopaque and the tumor site on the leg (arrowhead) has no radiopaque material.



**Figure 4:** Photograph of leg and resected tumor shown in Figure 3. Note the black dye in the tumor at the time of resection.



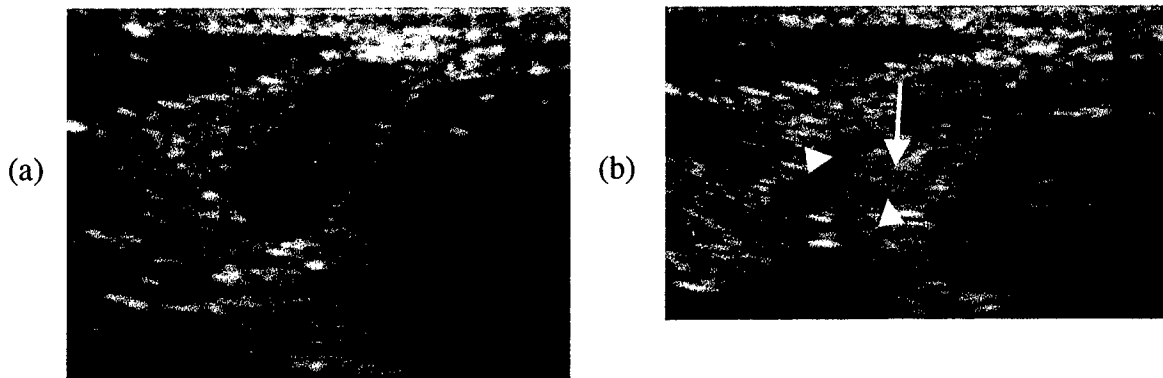
**Figure 5:** CT of the popliteal region demonstrating enhancement of a portion of the ipsilateral node (arrow).



**Figure 6:** CT of the retroperitoneum demonstrates enhancement of an ipsilateral iliac node (arrow).



**Figure 7:** Ultrasound scan of a popliteal node obtained before (a) and 24 hours (b) following the injection of PFC emulsion in the tumor. Note the enhancement of the center of the node (arrow) leaving the marginal sinus unenhanced (arrowheads). This was shown to be due to the accumulation of the agent in lymph node macrophages.





DEPARTMENT OF THE ARMY  
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND  
504 SCOTT STREET  
FORT DETRICK, MARYLAND 21702-5012

REPLY TO  
ATTENTION OF:

MCMR-RMI-S (70-1y)

8 Jan 2003

MEMORANDUM FOR Administrator, Defense Technical Information  
Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,  
VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to the enclosed. Request the limited distribution statement for the enclosed be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART  
Deputy Chief of Staff for  
Information Management

ADB265840

ADB266633

ADB282069

ADB279138

ADB251763

ADB265386

ADB264578

ADB281601

ADB282057

ADB281679

ADB258874

ADB258251

ADB281645

ADB281773

ADB264541

ADB261128

ADB281660

ADB241630

ADB261339

ADB259064

ADB281924

ADB273096

ADB266141

ADB281663

ADB281681

ADB281664

ADB281659

ADB259637

ADB258830

ADB256645

ADB266029

ADB262441

ADB281668

ADB281674

ADB259834

ADB281771

ADB266075

ADB281612

ADB281661